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Laboratory Evaluation of the Molluscicidal Activity of Calcium Hypochlorite on Intermediate Hosts of Paragonimiasis

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Abstract:
A laboratory investigation was conducted to assess the molluscicidal effect of calcium hypochlorite on two species of Potadoma snails: Potadoma moerchi and Potadoma freethi. 90 snails per species were collected from a stream in Ipogun village, Ondo State, Nigeria. Five (5) concentrations of calcium hypochlorite (45mg/l, 65mg/l, 85mg/l, 105mg/l, and 125mg/l) were prepared for the experiment using well water. The snails were then exposed to the different concentrations of the calcium hypochlorite solution in glassware for 48 hours with 3 replicates per treatment in a completely randomized design to determine the LC_{50} and LC_{90}. A control treatment was also set up for the experiment using well water. The results showed that Calcium hypochlorite had a remarkable molluscicidal activity on the snails, with a 93.33% percentage mortality rate recorded in Potadoma moerchi at 48 hours and 100% recorded from 32 hours for Potadoma freethi snails in the two highest concentrations. After the 48-hour assay, the mean LC_{50} and LC_{90} for P. moerchi were 91.42mg/l and 126.27mg/l, while the mean LC_{50} and LC_{90} for P. freethi were 51.98mg/l and 94.33mg/l. Calcium hypochlorite demonstrated effective molluscicidal activity against both snails and may be useful in controlling intermediate hosts of paragonimiasis, and its deployment in slow-flowing or stagnant water bodies will render it safe for communal use, particularly in areas where potable water is lacking in rural settlements.

Keywords: *Calcium hypochlorite, control, molluscicide, snail hosts, paragonimiasis*

1. Introduction

Freshwater snails (family: Planorbidae) are intermediate hosts for several snail-borne diseases of medical and veterinary importance (Akande & Odetola, 2011), and according to a taxonomic review, there are approximately 4,000 species of freshwater snails (Strong *et al*., 2008). It has been estimated that there are less than 350 species of intermediate hosts, or freshwater snails, in Africa alone (Yves *et al.,* 2013). In tropical and subtropical areas, parasitic diseases spread by freshwater snails, such as schistosomiasis, paragonimiasis, and fascioliasis, pose a considerable risk to people and animals and have a significant negative socio-economic impact. Freshwater snails are also an intermediate host for foodborne fluke infections that affect the liver, lungs, and intestines of humans or animals. Examples are *Fasciola* and *Paragonimus*. The trematode *Paragonimus* (lung fluke) causes human paragonimiasis, which can infect extra pulmonary areas such as the thorax, belly, skin, brain, spinal cord, and other unspecified organs and tissues in addition to the lungs. *P. westermani, P. africanus, P. heterotremus, P. kellicotti, P. mexicanus, P. siamensis, P. skrjabini, P. skrjabini miyazakii,* and *P. uterobilateralis* are the nine species that primarily cause human infections, and the majority of instances occur in Asia, Africa, and North and South America (Narain *et al.,* 2010; Chai, 2013). Controlling different species of freshwater snails is well-known to be an effective measure to reduce disease prevalence. Three main techniques have been carried out for managing intermediate hosts of trematode parasites: biological, ecological, and chemical control methods. The current tactics for controlling snail-borne parasitic diseases focus mostly on snail control, drug therapy, advanced sanitation, and health education, which are effective strategies for reducing the prevalence of the diseases. Chemical control of snail hosts is accomplished using compounds commonly known as molluscicides, and this control of snail host is based on the idea that the snail is the weakest link in the parasite life cycle and hence offers the most effective and rapid method of stopping the transmission cycle (Ritchie, 1973).

Calcium hypochlorite and *Tetrapleura tetraptera* have been shown to kill other snail hosts and their egg masses (Oniya *et al*., 2006; Oniya & Fajiwe, 2010; Oniya *et al*., 2013). However, the effects on the environment and other non-target organisms remain a concern (Ekabo *et al.,* 1996). Since they also kill fish and other animals and are used in endemic areas in water, which is often the only source for humans and their pets, preliminary and expansion tests are necessary to confirm their safety before approving their widespread use most

especially today when there is a far greater awareness of both toxicological and environmental risks (Sturrock, 1995). However, using calcium hypochlorite in the treatment of domestic, municipal water may be an advantage for its adoption as a safe molluscicide if effective at concentrations not above that permitted in treated water for human use. Therefore, research into more effective, affordable, and ecologically friendly chemicals with good molluscicidal characteristics is necessary. This study aims to investigate the potential or otherwise of calcium hypochlorite as a promising molluscicide against snail intermediate hosts of *Paragonimus* species. This study will also help public health personnel plan effective chemical control programs against freshwater snails, reducing the prevalence of snail-borne diseases in endemic areas.

2. Materials and Methods

All snails were collected using a scoop net and transported with source water to the Parasitology Laboratory, Biology Department, Federal University of Technology, Akure, Ondo State, where they were washed, sorted, and counted. Identification was made using standard morphometric identification keys (Brown & Kristensen, 1993). 90 *Potadoma moerchi* snails were distributed evenly into 6 glass tanks containing well water. Also, 90 *Potadoma freethi* were distributed evenly into another 6 glass tanks containing well water. The different snail species were given a week to acclimatize to the laboratory environment. During this time, they were fed dried lettuce every day, and no mortality was recorded throughout the period. Solutions of calcium hypochlorite (KEM Light Labs. PVT Ltd, India) were prepared in five concentrations of 45 mg/l, 65 mg/l, 85 mg/l, 105 mg/l, and 125 mg/l in different 1L bottles. The solutions were allowed to stand for six hours to ensure dissolution and homogeneity. After six hours, 400mL of each test solution was introduced into different 500mL glassware, and 5 snails of each species were introduced and observed over a 48h period. A control tank with only well water was also set up with the same number of snails. Each concentration of the test solutions was replicated three (3) times. Both control and treatment were set up for 48-hour and mortality assessments were done every four hours without feeding. After confirmation, dead snails were removed from the glassware.

3. Data Analysis

Over 48 hours, mortality rates were measured. Means and standard errors of means were determined, and Means were separated using Duncan's Multiple Range Test. Probit analysis was done to calculate the Mean Lethal concentration (LC_{50} and LC_{90}). Statistical Package for Social Science (SPSS) 20.0 was used for regression analysis to demonstrate the spatial association between the percentage death rates of the two species and the various treatment concentrations used.

4. Results

The response *of Potadoma moerchi* to the various concentrations of calcium hypochlorite was assessed every 4 hours for 48 hours (Table 1). At first, all snails withdrew into their shells, but as time progressed, the movement was observed in tanks with lower concentrations but slower than those in the control tank. All snails in two (2) replicates of the 125mg/l experiment were dead by 48 hours, with the first mortality occurring at 32 hours in replicate 2.

Concentrations (mg/l)	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs	28hrs	32hrs	36hrs	40hrs	44hrs	48hrs
45 (Replicate 1)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 2)	0	0	0	0	0	0	0	0	0	0	0	1
(Replicate 3)	0	0	0	0	0	0	0	0	0	0	0	0
65 (Replicate 1)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 2)	0	0	0	0	0	0	0	0	0	0	0	1
(Replicate 3)	0	0	0	0	0	0	0	0	0	0	0	1
85 (Replicate 1)	0	0	0	0	0	0	0	0	0	0	0	1
(Replicate 2)	0	0	0	0	0	0	0	0	0	0	1	2
(Replicate 3)	0	0	0	0	0	0	0	0	0	0	1	1
105 (Replicate 1)	0	0	0	0	0	0	0	0	1	2	4	4
(Replicate 2)	0	0	0	0	0	0	0	0	1	3	3	3
(Replicate 3)	0	0	0	0	0	0	0	0	1	1	3	4
125 (Replicate 1)	0	0	0	0	0	0	0	0	1	3	4	5
(Replicate 2)	0	0	0	0	0	0	0	1	2	2	3	4
(Replicate 3)	0	0	0	0	0	0	0	0	1	4	4	5
0 (Replicate 1)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 2)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 3)	0	0	0	0	0	0	0	0	0	0	0	0

Table 1: Mortality of Potadoma Moerchi in Response to Calcium Hypochlorite at Different Hours

The effect of calcium hypochlorite on snails maintained in solutions containing 45 mg/l, 65 mg/l, and 85 mg/l was not significantly different ($P>0.05$) (Table 2) from the control while those maintained in solutions containing 105 mg/l and 125 mg/l differed significantly ($P<0.05$) at 36 hours from those maintained in the control tank and the other solutions. The mortality rate with the highest percentage was found to be 125 mg/l (93.33%), followed by 105 mg/l (73.33%). The mortality rate was the same (13.33%) at 65 mg/l and 85 mg/l. The snails in the control glassware were active, and no mortality was observed throughout the experiment. Dead snail shells developed a clear whitish shell coating.

Mean followed by the same alphabet in column are not significantly different from one another ($P>0.05$) using Duncan Multiple Range Test (DMRT).

After 36 hours of exposure to the snails, the effects of the test solution at various doses revealed varying toxicity levels (Table 3). The lethal concentrations of LC_{50} and LC_{90} were 91.42 mg/l and 126.27 mg/l, respectively. Additionally, the regression model demonstrated a perfect correlation between the snails' reaction to calcium hypochlorite as R = 1.0 (36 and 40 hours) and 0.79. (44 hours). Also, LC_{50} and LC_{90} decreased with increasing exposure time, indicating that the longer these snails were exposed to molluscicide, the greater the likelihood of successful control.

Over the course of 48 hours, mortality of *Potadoma freethi* was assessed every 4 hours in the different concentrations (Table 4). Except for the snails in the control tanks, all snails in the other test solutions withdrew back into their shells immediately after exposure. All snails in all replicates of the 125mg/l experiment, two (2) replicate of the 105mg/l experiment, and one (1) replicate of the 85mg/l experiment was dead by 48 hours. The first mortality occurred at 16 hours in one (1) replicate of 85mg/l, 12 hours in one (1) replicate of 105mg/l concentration, and 8 hours in the two (2) replicates of 125mg/l.

	Period of exposure (%Mortality ± S.E)				
Conc. (mg/l)	**32 hours**	**36 hours**	**40 hours**	**44 hours**	**48 hours**
45	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
65	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	13.33±6.67^a
85	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	13.33±6.67^a	13.33±6.67^a
105	0.00±0.00^a	20.00±0.00^b	40.00±11.55^b	66.67±6.67^b	73.33±6.67^b
125	6.67±6.67^a	26.67±6.67^b	60.00±11.55^b	66.67±6.67^b	93.33±6.67^c
Control	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a

Table 2: Mean Mortality of P. morechi after 48hrs in Different Concentrations

Time (hrs)	**Intercept**	**Slope**	**LC_{50} (LCL -UCL)**	**LC_{90} (LCL -UCL)**	**R^2**	**Logistic (Log_{10}) probit regression equation**
36	1.68	2.89	205.33 (143.29 –294.23)	570.13 (397.87 – 816.96)	1.00	y = 2.8895x – 1.6819
40	8.78	6.69	114.56 (98.45 – 133.32)	178.06 (153.01 – 207.21)	1.00	y = 6.6916x – 8.7784
44	14.17	9.47	106.00 (94.67 – 118.69)	145.96 (130.35 – 163.43)	0.79	y = 9.47x – 14.173
48	14.32	9.82	91.42 (82.03 – 101.87)	126.27 (115.01 – 140.71)	0.85	y = 9.8204x – 14.323

Table 3: LC_{50} and LC_{90} of Calcium Hypochlorite on P. morechi

Note: R^2 = Statistical measure of mortality proportion in the regression model
LC_{50} = Lethal concentration at which 50% population respond
LC_{90} = Lethal concentration at which 90% population respond
LCL = Lower confidence limit
UCL = Upper confidence limit

Concentrations (mg/l)	**4hrs**	**8hrs**	**12hrs**	**16hrs**	**20hrs**	**24hrs**	**28hrs**	**32hrs**	**36hrs**	**40hrs**	**44hrs**	**48hrs**
45 (Replicate 1)	0	0	0	0	0	0	0	1	1	2	2	3
(Replicate 2)	0	0	0	0	0	0	0	0	1	1	1	2
(Replicate 3)	0	0	0	0	0	0	0	0	0	1	1	1
65 (Replicate 1)	0	0	0	0	0	1	1	2	2	3	3	3
(Replicate 2)	0	0	0	0	0	0	1	1	3	4	4	4
(Replicate 3)	0	0	0	0	0	0	1	2	2	2	3	3
85 (Replicate 1)	0	0	0	2	2	3	3	4	4	5	5	5
(Replicate 2)	0	0	0	0	1	1	2	3	3	4	4	4
(Replicate 3)	0	0	0	0	1	2	2	3	3	3	3	4
105 (Replicate 1)	0	0	1	2	3	3	3	4	4	4	4	4
(Replicate 2)	0	0	0	1	1	2	2	3	5	5	5	5
(Replicate 3)	0	0	0	1	1	2	3	3	4	4	4	5
125 (Replicate 1)	0	2	3	3	3	4	4	5	5	5	5	5
(Replicate 2)	0	1	2	3	3	3	4	5	5	5	5	5
(Replicate 3)	0	0	2	3	3	4	5	5	5	5	5	5
0mg/l (Replicate 1)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 2)	0	0	0	0	0	0	0	0	0	0	0	0
(Replicate 3)	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Mortality of Potadoma freethi in Response to Calcium Hypochlorite at Different Hours

The effect of calcium hypochlorite on snails maintained in all solutions was significantly different ((P<0.05) from the control (Table 5). The mortality rate with the highest percentage was found to be 125mg/l (100%), followed by 105mg/l (93.33%), 85mg/l (86.67%), 65mg/l (66.67%), and 45mg/l (40.00%). The snails in control were active, and no

mortality was observed throughout the experiment. After 36 hours of exposure to the snails, the effects of the test solution at various doses revealed varying toxicity levels (Table 6). The lethal concentration 50 (LC_{50}) and 90 (LC_{90}) were 51.98 mg/l and 94.33 mg/l, respectively. The highest toxicity effect was observed at 48 hours, and the mortality proportion in the regression model was 0.99, suggesting a perfect correlation between the snails and the test solution (Table 6). Additionally, R = 0.99 (36 and 40 hours) and 0.96 (44 hours) from the regression model showed a perfect connection between the snails' response to calcium hypochlorite (48 hours). The LC_{50} and LC_{90} values also decreased with longer exposure times, showing that the longer these snails were exposed to molluscicide, the higher the possibility of effective control.

Conc.	Period of Exposure (%Mortality ± S.E.)										
	8 hours	**12 hours**	**16 hours**	**20 hours**	**24 hours**	**28 hours**	**32 hours**	**36 hours**	**40 hours**	**44 hours**	**48 hours**
45	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.67±6.67^{a}	13.33±6.67^{a}	26.67±6.67^{b}	26.67±6.67^{b}	40.00±11.55^{b}
65	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.67±6.67^{a}	20.00±0.00^{b}	33.33±6.67^{b}	46.67±6.67^{b}	60.00±11.55^{c}	66.67±6.67^{c}	66.67±6.67^{c}
85	0.00±0.00^{a}	0.00±0.00^{a}	13.33±13.33^{ab}	26.67±6.67^{b}	33.33±17.64^{b}	46.67±6.67^{c}	66.67±6.67^{c}	66.67±6.67^{c}	80.00±11.55^{cd}	80.00±11.55^{cd}	86.67±6.67^{cd}
105	0.00±0.00^{a}	6.67±6.67^{a}	26.67±6.67^{b}	33.33±17.64^{b}	40.00±0.00^{b}	53.33±6.67^{c}	66.67±6.67^{c}	86.67±6.67^{d}	86.67±6.67^{d}	86.67±6.67^{cd}	93.33±6.67^{d}
125	20.00±11.55^{b}	46.67±6.67^{b}	60.00±0.00^{c}	60.00±0.00^{c}	73.33±6.67^{c}	86.67±6.67^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Table 5: Mean Mortality of P. Freethi after 48hrs in Different Concentrations

Mean followed by the same alphabet in column are not significantly different from one another (P>0.05) using Duncan Multiple Range Test (DMRT)

Time (hours)	Intercept	Slope	LC_{50} (LCL -UCL)	LC_{90} (LCL -UCL)	R^2	Logistic (Log_{10}) Probit Regression Equation
36	5.81	5.88	69.11 (58.87 – 81.13)	114.22 (97.29 – 134.08)	0.99	y = 5.8783x – 5.8126
40	3.52	4.81	59.15 (48.69 – 71.86)	109.49 (90.14 – 133.04)	0.99	y = 4.8063x – 3.5159
44	3.29	4.71	57.65 (47.23 – 70.37)	108.25 (88.68 – 132.13)	0.96	y = 4.709x – 3.292
48	3.31	4.86	51.98 (41.83 – 63.09)	94.33 (76.80 – 115.85)	0.99	y = 4.8565x – 3.3075

Table 6: LC_{50} and LC_{90} of Calcium Hypochlorite on P. freethi

Note: R^2 = Statistical measure of mortality proportion in the regression model
LC_{50} = Lethal concentration at which 50% population response
LC_{90} = Lethal concentration at which 90% population response
LCL = Lower confidence limit
UCL = Upper confidence limit

5. Discussion

Potadoma species are intermediate hosts for paragonimiasis (Aka *et al*., 2008), and the aquatic snail *Potadoma freethi* is known to be the first intermediate host for *Paragonimus africanus*. Most snail-borne trematodes are currently controlled primarily through vector eradication, medication therapy, better cleanliness, and health education (Limpanont *et al*., 2020). However, to stop the spread of these invasive species, snail control employing molluscicides still plays a significant role and is an important method (Wang *et al.,* 2009; Zheng *et al.,* 2021). This study showed that calcium hypochlorite was toxic to *Potadoma moerchi* and *Potadoma freethi* snails, establishing intermediate hosts for paragonimiasis. More than 70% of the snails exposed to the solution died within 48 hours, demonstrating the solution's toxicity on *P. moerchi* snails at the highest concentrations (105 mg/l and 125 mg/l). The mortality rate was less than 14% in other concentrations, which was comparatively low. In all concentrations, calcium hypochlorite had a substantially distinct molluscicidal effect on *P. freethi* snails at different time intervals, and a 100% mortality rate at 32 hours was observed in the 125 mg/l concentration. Four concentrations (65 mg/l, 85 mg/l, 105 mg/l, and 125 mg/l) showed mortality rates of about 60% at 48 hours with significant toxicities of up to 80% at 85 mg/l, 105 mg/l and 125 mg/l. This demonstrates that *P. freethi* snails were more sensitive to calcium hypochlorite than *P. moerchi* snails. The mean LC_{50} and LC_{90} after the 48-hour assay were 91.42 mg/l and 126.27 mg/l for *P. morechi* and 51.98 mg/l and 94.33 mg/l *for P. freethi*. Oniya (2019), who validated that the test chemical displayed an excellent piscicidal effect on fingerlings of *Clarias gariepinus*, also confirmed the potency of this test solution. To destroy hazardous bacteria, Piper *et al.* (1998) reported that an application of 5–10 mg/L chlorine for 1–24 hours is sufficient; however, the precise dosage depends on the target species and the local water chemistry, notably the presence of organic matter. The cause of death observed due to the snails' exposure to calcium hypochlorite is consistent with Olasehinde (2000), which stated that very high quantities are harmful to snail intermediate hosts and also affect the eggs' capacity to hatch. The most plausible mechanism of toxicity is that severe alterations in water chemistry directly harm most aquatic creatures' gills and impair breathing.

6. Conclusion

The findings demonstrate that Calcium hypochlorite at low concentrations had a significant impact on *Potadoma* species mortality, hence, can be deployed in molluscicidal programs because of its proven efficiency in the laboratory. Also, the advantage of this compound over others is owing to the chemical's versatility, as it can be used to sterilize swimming pools and disinfect water bodies in general due to its high chlorine availability. It can also be used to disinfect huge amounts of water to make it safe for drinking. Routine health education and focal group discussions should be carried out in the community to educate the population on the inherent danger of the presence of snails in their waters. Also, the community should own this control initiative, while the government should provide the required infrastructural support to prevent the outbreak of paragonimiasis in the community rather than wait to mount control programs after an outbreak.

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